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Nanotechnology and Its Applications in Medical Diagnosis

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ABSTRACT

Nanobiotechnology and its applications in life sciences particularly in molecular diagnostics are termed as Nanomolecular diagnostics, which offers new options for clinical diagnostic procedures. Molecular diagnostics is an essential part of the development of personalized medicine, which features point-of-care performance of diagnostic procedure. Nanobiotechnology incorporated along with molecular diagnostics improves the clinical diagnosis tremendously. In this type of diagnosis, 'Nanoscale probes' are mostly suitable for detailed analysis of receptors, pores and other components of living cells that exist in nanoscale dimension. For combined diagnosis and therapeutics, Nanodevice can be implanted as a preventive and prophylactic measure in early disease diagnosis. Advantages of applying nanotechnology to molecular diagnostics are that only small amounts of sample material are needed and that diagnostic tests that use nanoscale particles as tags or labels are faster and more sensitive [1].

KEY WORDS: Nanobiotechnology – Nanobiochips – Nanosensors – Quantum Dots – Nanobiosensors – Nanobarcodes - Carbon nanotubes – Nanopores.

INTRODUCTION

Nanotechnology is the creation and utilization of materials, devices, and systems through the control of matter on the nanometer (1 billionth of a meter)-length scale and its application in life sciences is termed as 'nanobiotechnology' [2]. Nanotechnology is often represented by two fundamentally different approaches: 'top-down' and 'bottom-up'. 'Top-down' refers to making nanoscale structures by machining, templating and lithographic techniques, whereas 'bottom-up', or molecular nanotechnology, applies to building organic and inorganic materials into defined structures, atom-by-atom or molecule by- molecule, often by self-assembly or self-organisation. Biologists/chemists are involved in the synthesis of inorganic, organic and hybrid nanomaterials for the use in nanodevices, the development of novel nanoanalytical techniques. Nanodiagnostics technologies like Nanoscale visualization, Nanoparticle biolabels, biochips/microarrays, Nanoparticle-based nucleic acid diagnostics, Nanoproteomic-based diagnostics, Biobarcode assays, Nanopore technology, DNA nanomachines, Nanoparticle-based immunoassays, Nanobiosensors etc., are growingly popular in the field of medical diagnostics [3].

Nanotechnology-Based Biochips and Microarrays

Generally the cell constituents exists in nano size and also the technology which is used to monitor/diagnose these biological molecules are also falls in nano scale dimension which can be regarded as 'Nanotechnology on a chip' is a new paradigm for total chemical analysis systems [4]. Some examples of devices that incorporate nanotechnology-based biochips and microarrays are nanofluidic arrays and protein nanobiochips. These chips can be designed to interact with cellular constituents with higher specificity [5]. One of the more promising uses of nanofluidic devices is isolation and analysis of individual biomolecules, such as DNA. This capability could lead to new detection schemes for cancer. One such device entails the construction of silicon nanowires on a substrate, or chip, using standard photolithographic and etching techniques, followed by a chemical oxidation step that converts the nanowires into hollow nanotubes [2]. With this process the investigators can reliably create nanotubes with diameters as small as 10 nm, although devices used for biomolecule isolation contain nanotubes with diameters of 50 nm. Trapping DNA molecules requires a device consisting of a silicon nanotube connecting 2 parallel microfluidic channels. Electrodes provide a source of current used to drive DNA into the nanotubes. Each time a single DNA molecule moves into the nanotube, the electrical current suddenly changes. The current returns to its baseline value when the DNA molecule exits the nanotube. Nanofluidic technology is expected to have broad applications in systems biology, personalized medicine, pathogen detection, drug development, and clinical research.

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Protein Microarrays/Chips

Generally 2-D gel electrophoresis and mass-spectrometric methods (sensitivity to femtomole-attomole protein concentration) are used as the central tools for protein identification and characterization between healthy and diseased samples. In addition to the above methods, protein microarray offer a powerful tool for screening thousands of proteins at a time, where variety of proteins such as antibodies and enzymes are immobilized in an array format on glass slide by robots. The surface of the glass slide is then probed with sample of interest that binds to relevant antibodies on chip which will be analysed by relevant detection method on array. Refinement in microarrays miniaturization (with the advent of nanotechnology) will further contribute to molecular diagnostics and the development of personalized medicine [6]. Profiling proteins on arrays will be used in distinguishing the proteins of normal cells from early stage cancer cells and malignant metastatic cancer cells [7]. It is also applicable to study the protein-protein interaction, the functional activity of proteins and in the discovery of disease markers and diagnosis [8, 9]. It can also be used for profiling of the serum to differentiate patients with pancreatic cancer from those with other pancreatic diseases and from healthy control [10].

Heart failure, arising from systemic disease or specific heart muscle disease, is one of the leading causes of morbidity and mortality in developing countries. Proteomics based approach in characterizing overall changes in protein expression in heart diseases may provide new insights into the cellular involvement of interactions [11]. Replacement of growing blood vessels in heart attack will be an addition to nanotechnology. The endothelial cells harvested in silicon molds shapes like capillaries; these cultivars would be delivered to the heart via microscopic machines called 'Angiochips' which will repair the damage caused by heart attack [12]. Thus, protein microarrays offer the possibility of developing a rapid global analysis of the entire proteome, leading to protein-based diagnostics and therapeutics. It has been successfully applied to the study of complex trait i.e. cardiovascular diseases, cancer and type II diabetes. Recent development in microarrays has come with technique called "Immunosensing", where patterned microarrays of antibodies to specific bacteria were used to perform a series of bacterial immunoassay and characterized using scanning probe microscopy

Protein chips, based on protein-binding silica-nanoparticles, are in developmental stage. The surface of a silica-nanoparticle with a diameter of less than 100 nm can be configured with many different capture proteins. The particles configured in this way are then applied to silicon carriers in thin, even layers.After contact is made with a sample, the chips can be analyzed using state-of-the art mass spectrometry (MS)–Matrix-Assisted Laser Desorption/Ionization (MALDI)–time-of-flight (TOF) MS [4].

Nanobiosensors

Biosensors are chemical sensors, in which recognition processes rely on biochemical mechanisms utilisation. They consist of a biological element (responsible for sampling), and a physical element (often called transducer, transmitting sampling results for further processing) [13]. Nanomaterials are exquisitely sensitive chemical and biological sensors [14]. Ability to identify a particular type of cells or areas in a body makes the nanobiosensors to find its place in medical diagnostics. Based on the differences in volume, concentration, displacement and velocity, gravitational, electrical, and magnetic forces, pressure, or temperature of cells in a body, nanosensors may be able to distinguish between and recognize certain cells, most notably those of cancer, at the molecular level in order to deliver medicine or monitor development to specific places in the body. In addition, they may be able to detect macroscopic variations from outside the body and communicate these changes to other nanoproducts working within the body.

One example of nanosensors involves using the fluorescence properties of cadmium selenide quantum dots as sensors to uncover tumors within the body. By injecting a body with these quantum dots, a doctor could see where a tumor or cancer cell was by finding the injected quantum dots, an easy process because of their fluorescence. Developed nanosensor quantum dots would be specifically constructed to find only the particular cell for which the body was at risk. A downside to the cadmium selenide dots, however, is that they are highly toxic to the body. As a result, researchers are working on developing alternate dots made out of a different, less toxic material while still retaining some of the fluorescence properties. In particular, they have been investigating the particular benefits of zinc sulfide quantum dots which, though they are not quite as fluorescent as cadmium selenide, can be augmented with other metals including manganese and various lanthanide elements.

In addition, these newer quantum dots become more fluorescent when they bond to their target cells. (Quantum) Potential predicted functions may also include sensors used to detect specific DNA in order to recognize explicit genetic defects, especially for individuals at high-risk and implanted sensors that can automatically detect glucose levels for diabetic subjects more simply than current detectors. [15]. DNA can also serve as sacrificial layer for manufacturing CMOS IC, integrating a nanodevice with sensing capabilities. Therefore, using proteomic patterns and new hybrid materials, nanobiosensors can also be used to enable components configured into a hybrid semiconductor substrate as part of the circuit assembly. The development and miniaturization of nanobiosensors should provide interesting new opportunities.

Other projected products most commonly involve using nanosensors to build smaller integrated circuits, as well as incorporating them into various other commodities made using other forms of nanotechnology for use in a variety of situations including transportation, communication, improvements in structural integrity, and robotics. Nanosensors may also eventually be valuable as more accurate monitors of material states for use in systems where size and weight are constrained, such as in satellites and other aeronautic machines. [1].

Nanoscale Single-Cell or Molecule Identification

Nanotechnology has facilitated the development of methods for detection of single cells or a few molecules. Nanolaser scanning confocal spectroscopy, with the capability of single-cell resolution, can be used to identify previously unknown properties of certain cancer cells that distinguish them from closely related nonpathogenic cells [16]. Nanoproteomics, the application of nanobiotechnology to proteomics, can enable detection of a single molecule of protein. Biobarcode assays enable detection in body fluids of miniscule amounts of proteins that cannot be detected by conventional methods [1]. A 2-dimensional method for mass spectrometry in solution is based on the interaction between a nanometer-scale pore and analytes [9]. An applied electric current is used to force charged molecules (such as single-stranded DNA) one at a time into the nanopore, which is only 1.5 nm at its smallest point. As the molecules pass through the channel, the current flow is reduced in proportion to the size of each individual chain, allowing its mass to be easily derived. This single-molecule analysis technique could prove useful for the real-time characterization of biomarkers.

Application of Nanoparticles for Discovery of Biomarkers

Currently available molecular diagnostic technologies have been used to detect biomarkers of various diseases. Nanotechnology has refined the detection of biomarkers. Some biomarkers also form the basis of innovative molecular diagnostic tests. The physicochemical characteristics and high surface areas of nanoparticles make them ideal candidates for developing biomarker-harvesting platforms. Given the variety of nanoparticle technologies that are available, it is feasible to tailor nanoparticle surfaces to selectively bind a subset of biomarkers and sequester them for later study using high-sensitivity proteomic tests [17, 18]. Biomarker harvesting is an underutilized application of nanoparticle technology and is likely to undergo substantial growth. Functional polymer-coated nanoparticles can be used for quick detection of biomarkers and DNA separation.

Nanoparticles for Molecular Diagnostics

Several nanoparticles have been used for diagnostics. Of these, the most frequently used are gold nanoparticles, QDs, and magnetic nanoparticles.

Gold Nanoparticles for Diagnostics

Small pieces of DNA can be attached to gold particles no larger than 13 nm in diameter. The gold nanoparticles assemble onto a sensor surface only in the presence of a complementary target. If a patterned sensor surface of multiple DNA strands is used, the technique can detect millions of different DNA sequences simultaneously. The current non-optimized detection limit of this method is 20 fmol/L. Gold nanoparticles are particularly good labels for sensors because a variety of analytical techniques can be used to detect them [18].

Quantum Dots

QDs are inorganic fluorophores that offer significant advantages over conventionally used fluorescent markers. Inorganic crystals of CdSe (cadmium selenide 200-10000 atoms wide), coated with ZnS (zinc sulphide). They emit fluorescent light when irradiated with low energy light. The size of the dots (< 10 nm) determines the frequency of light emitted (i.e. colour). The dots usually have a polymer coating with multivalent bio-conjugate attached, or are embedded into microbeads. Collection of dots of different size embedded to a given microbead emits distinct spectrum of colours - spectral bar code specific for this bead. Detection technique with the use of 10 intensity levels and 6 colours could theoretically provide 106 distinct codes. Quantum dots, for example CdSe-ZnS nanocrystals, do no emit in the near infrared, so they cannot be used for analysis in blood [13,19].

They have high sensitivity, broad excitation spectra, stable fluorescence with simple excitation, and no need for lasers. Their red/infrared colors enable whole blood assays. QDs have a wide range of applications for molecular diagnostics and genotyping. QDs also enable multiplexed diagnostics and integration of diagnostics with therapeutics. The most important potential applications of QDs are for cancer diagnosis. Luminescent and stable QD bioconjugates enable visualization of cancer cells in living animals. QDs can be combined with fluorescence microscopy to follow cells at high resolution in living animals. QDs have been coated with a polyacrylate cap and covalently linked to antibodies for

immunofluorescent labeling of breast cancer marker Her2. Carbohydrate-encapsulated QDs with detectable luminescent properties are useful for imaging of cancer. QDs can be used in multicolour optical coding for biological assays [20].

Nanobarcodes

Freestanding, cylindrical nanoparticles with specific patterns of submicron stripes of noble metal ions, produced by alternating electrochemical reduction of the appropriate metals with the dimensions between 12 nm and 15 μm in width and 1–50 μm in length. The differential reflectivity of the adjacent metals provides contrast black and white stripes which makes them distinctive (like conventional barcodes) under light, or fluorescent microscopy, or mass spectrometry [21]. Nanobarcodes are useful in SNP mapping, Coding in multiplexed assays for proteomics, population diagnostics and in point-of-care handheld devices. Proteins detection by either mass spectrometry or fluorescence measure (after proteins immobilization on a metal surface) [13].

Magnetic Nanoparticles

Iron nanoparticles, 15–20 nm in size and having saturation magnetization, have been synthesized and embedded in copolymer beads of styrene and glycidyl methacrylate (GMA), which were coated with polyGMA by seed polymerization [22]. The resulting Fe/St-GMA/GMA beads had diameters of 100 –200 nm. Coating with polyGMA changed the zeta potential of the beads from 93.7 to 54.8 mV, as measured by an electrophoresis method. As revealed by gel electrophoresis, this process facilitates nonspecific protein adsorption suppression, which is a requisite for nanoparticles to be applied to carriers for bioscreening. Nanoparticles are used as labeling molecules for bioscreening. Superparamagnetic nanoparticles are useful for cell-tracking cells and for calcium sensing. Ferrofluids consist of a magnetic core surrounded by a polymeric layer coated with antibodies for capturing cells. A family of calcium indicators for MRI is formed by combining a powerful SPIO nanoparticle-based contrast mechanism with the versatile calcium-sensing protein calmodulin and its targets [23]. Superparamagnetic nanoparticles measuring 2–3 nm have been used in conjunction with MRI to reveal small and otherwise undetectable lymph-node metastases. Ultrasmall SPIO enhances MRI for imaging cerebral ischemic lesions. A dextran-coated iron oxide nanoparticle enhances MRI visualization of intracranial tumors for more than 24 h.

Nanoparticles as Biosensors Components

There are several kinds of nanoparticles that can be used as biosensors components. Most of them work as probes recognizing and differentiating an analyte of interest for diagnostic and screening purposes. In such applications biological molecular species are attached to the nanoparticles through a proprietary modification procedure. The probes are used then to bind and signal the presence of a target in a sample by their colour, mass, or other physical properties. The molecular binding is a subject of the biological surface science [24], which is closely related to the research on modification of nanostructures properties by controlling their structure and their surface at a nanoscale level [25,26]. Both fields cover broad area, in which one can locate the use of nanostructured platinum-lipid biolayer composite as biosensor [27] or research on endothelisation and adherence of the cells to nanostructure surfaces [28]. Biosensors based on quantum dots, nanobarcodes, metallic nanobeads, silica nanoparticles, magnetic beads, and carbon nanotubes can be qualified to the group of nanoprobes. The other biosensors employ nanoparticles in a different way. They work as sieves through which charged molecules are transported in an electrical field. Particles with engineered nanopores are used. But solid-state materials are not the only resources for nanoparticles construction. In example DNA-nanopores made of α-haemolysin protein channel mounted in a lipid bilayer with ~2 nm in diameter are made of organic material. These nanopores are able to discriminate between individual DNA strands up to 30 nucleotides in length, differing by a single base substitution. There are several articles concerning this subject, [29,30,31,32 and 33], while a comprehensive review is provided by [34]. They discuss native α - haemolysin sensor applications and present sensors based on modified α - haemolysin (together with description of other organic pores and synthetic nanopores, supporting structures and applications) [1].

Application of Nanodiagnostics in Infectious Diseases

The rapid and sensitive detection of pathogenic bacteria at the point of care is extremely important. Limitations of most of the conventional diagnostic methods are lack of ultrasensitivity and delay in getting results. A bioconjugated nanoparticle-based bioassay for in situ pathogen quantification can detect a single bacterium within 20-min [35]. Detection of single-molecule hybridization has been achieved by a hybridization-detection method using multicolor oligonucleotide-functionalized QDs as nanoprobes [36]. In the presence of various target sequences, combinatorial self-assembly of the nanoprobes via independent hybridization reactions leads to the generation of discernible sequence-specific spectral codings. This method can be used for genetic analysis of anthrax pathogenicity by simultaneous

detection of multiple relevant sequences. A spectroscopic assay based on SERS using silver nanorods, which significantly amplify the signal, has been developed for rapid detection of trace levels of viruses with a high degree of sensitivity and specificity [37]. The technique measures the change in frequency of a near- infrared laser as it scatters viral DNA or RNA. That change in frequency is as distinct as a fingerprint. This novel SERS assay can detect spectral differences between viruses, viral strains, and viruses with gene deletions in biological media. The method provides rapid diagnostics (60 s) for detection and characterization of viruses generating reproducible spectra without viral manipulation. This method is also inexpensive and easily reproducible.

Future Issues in the Development of Nanobiosensors

New biosensors and biosensor arrays are being developed using new materials, nanomaterials, and microfabricated materials and new methods of patterning. Biosensor components will use nanofabrication technologies. Nano sized devices can be produced by use of nanotubes, fullerenes (buckyballs), and silica and its derivatives. Some of the challenges will be development of real-time noninvasive technologies that can be applied to detection and quantification of biological fluids without the need for multiple calibrations using clinical samples. It would be desirable to develop multiple integrated biosensor systems that use doped oxides, polymers, enzymes, or other components to give the system the required specificity. Such integrated sensor systems would include all of the sensor components, software, plumbing, and reagents along with sample processing. There is also a need for reliable fluid handling systems for "dirty" fluids and for relatively small quantities of fluids (nanoliter to attoliter quantities). These should be low cost, disposable, reliable, and easy to use as part of an integrated sensor system. Sensing in pico liter to atto liter volumes might create new problems in the development of microreactors for sensing and novel phenomena in very small channels.

Safety Issues of Nanoparticles for Diagnostics

Potential toxic effects are a concern with in vivo use of nanoparticles but not with in vitro diagnostics, which forms the major portion of laboratory diagnostics. There are environmental concerns about the release of nanoparticles during manufacturing of nanoparticles and the environmental effects. These are being studied along with naturally present nanoparticles in the atmosphere. There are still many unanswered questions about the fate of nanoparticles introduced into the living body. Because of the huge diversity of materials used and the wide range in size of nanoparticles, these effects will vary considerably. QDs made with fluorescent labels of calcium selenide or zinc sulfide to increase stability may release potentially toxic cadmium and zinc ions into cells. Capping QDs with ZnO effectively prevents Cd_20 formation on exposure to air but not to ultraviolet radiation, and the search for better coating materials is ongoing. A high-throughput gene expression test determined that specially coated QD fluorescent nanoprobes affect only 0.2% of the human genome, dispelling the concern that the mere presence of these potentially toxic sentinels disrupts cell function [19]. It is conceivable that particular sizes of some materials may have a bearing on toxic effects Concern centers around nanoparticles smaller than 20 nm in diameter, which can penetrate the cells. One limitation for the approval of in vivo nanomaterials for human diagnostics would be that demonstration of safety of nanoparticles would be required. A number of studies have been done, but at this stage, no conclusions can be drawn about the safety of nanoparticles.

Future Prospects

Medical diagnosis, proper and efficient delivery of pharmaceuticals, and development of artificial cells are the medical fields where nanosize materials have found practical implementations. The application of nanotechnology to medicine, nanomedicine, subsumes three mutually overlapping and progressively more powerful molecular technologies [13]. First, nanoscale-structured materials and devices that can be fabricated today hold great promise for advanced diagnostics and biosensors, targeted drug delivery and smart drugs, and immunoisolation therapies. Second, biotechnology offers the benefits of molecular medicine via genomics, proteomics, and artificial engineered microbes. Third, in the longer term, molecular machine systems and medical nanorobots will allow instant pathogen diagnosis and extermination, chromosome replacement and individual cell surgery *in vivo*, and the efficient augmentation and improvement of natural physiological function [38].

Within the next decade, measurement devices based on nanotechnology, which can make thousands of measurements very rapidly and very inexpensively, will become available. Future trends in diagnostics will continue in miniaturization of biochip technology to the nanoscale range. Molecular electronics and nanoscale chemical sensors will enable the construction of microscopic sensors capable of detecting patterns of chemicals in a fluid. Estimates of plausible device capabilities have been used to evaluate their performance for typical chemicals released into the blood by tissues in response to localized injury or infection [39]. These indicate that the devices can readily enable differentiation of a single cell–sized chemical source from the background chemical concentration in vivo, providing high-resolution sensing in both time and space. With currently used methods for blood analysis, such a chemical source would be difficult to distinguish from background when diluted throughout the blood volume and withdrawn as a blood

sample. The trend will be to build the diagnostic devices from the bottom up, starting with the smallest building blocks. Whether interest and application of nanomechanical detection will hold in the long range remains to be seen. Another trend is to move away from fluorescent labeling as miniaturization reduces the signal intensity, but there have been some improvements making fluorescent labeling methods viable with nanoparticles.

Conclusion

Over coming years nanotechnology can be practically applied is creation of artificial cells, tissues and organs. Artificial cells are being actively investigated for use in the replacement of defective or incorrectly functioning cells and organs, especially related to metabolic functions. Nanotechnologies promise to extend the limits of current molecular diagnostics and enable point-of-care diagnosis, integration of diagnostics with therapeutics, and development of personalized medicine [40]. The most important clinical applications of currently available nanotechnology are in the areas of DNA detection assay, biomarker discovery, cancer diagnosis and detection of infectious microorganisms. Nanomedicine promises to play an important role in the future development of diagnostic and therapeutic methods.

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